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Micronization of Dihydroartemisinin by Rapid Expansion of Supercritical Solutions

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The purpose of this study was to prepare fine particles of antimalarial drug dihydroartemisinin (DHA) by rapid expansion of supercritical solutions (RESS) using carbon dioxide as supercritical fluid. The mechanical grinding by jet mill and additional vibration rod mill also was performed as a comparative method. In the RESS process, drug particles were prepared by varying processing conditions, including extraction condition, pre-expansion condition, nozzle diameter, nozzle temperature, and collecting distance. Particle size and morphology and physicochemical characteristics of the drug particles were investigated. The RESS process could produce the smaller drug particles (about 1–2 μm) when compared to mechanical grinding method (about 7 μm). All RESS processing parameters had an effect on size and morphology of drug particles. The particle size of drug was related to the solubility of drug in supercritical CO_2 at each processing condition. The fine particles of DHA (about 1 μm) with narrow size distribution could be obtained at extraction pressure of 18 MPa and extraction temperature of 32°C, which was closed to the critical temperature of supercritical CO_2 whereas broad size distribution was obtained at extraction temperature of 60°C. Powder X-ray diffraction study indicated that the RESS-processed particles were in crystalline form. The results revealed that RESS process is applicable for micronization of DHA.

Keywords micronization; dihydroartemisinin; supercritical fluid; rapid expansion of supercritical solutions; RESS

INTRODUCTION

Most drugs are insoluble or poorly soluble in water. Improvement of therapeutic performance of these hydrophobic drugs is one of the challenges in pharmaceutical product development. Many strategies are employed to improve solubility of drugs, such as particle size reduction, use of surfactant, use of co-solvent, complex formation, and solid dispersion. Grinding (milling) is a major technique used to reduce particle size of drugs. However, heat- or mechanical stress-induced degradation of drugs often takes place. Furthermore, the size distribution is usually broad and hardly reaches to micron or sub-micron level. Recrystallization is another technique used to prepare drug fine particles. This technique has some disadvantages such as a large amount of organic solvent used and remaining solvent residues.

Recently, supercritical fluid technology, regarded as a green technology, has been used for micronization of drugs (Fages et al., 2004; Helfgen et al., 2001; Hu, Johnston, & Williams III, 2004; Jung & Perrut, 2001; York, 1999; Young et al., 2000). Supercritical fluids have several advantageous properties due to high density, low viscosity, and high diffusion rate (Budich & Brunner, 2003). As a supercritical fluid, carbon dioxide (CO_2) has been widely used due to its mild critical temperature and pressure (31.1ºC and 7.39 MPa) and appreciable solubilizing power for many organic compounds. Supercritical CO_2 is advantageous to the environment due to its non-toxic and easily recycled properties. In addition, CO_2 is inexpensive and nonflammable. Therefore, supercritical CO_2 has gained much interest in heat-sensitive and contaminant-free pharmaceutical processing (Fages et al., 2004; Shekunov & York, 2000).

In the pharmaceutical industry, methods for particle formation in supercritical fluids are rapid expansion of supercritical solutions (RESS), supercritical antisolvent process (SAS), gas antisolvent (GAS), aerosol solvent extraction system (ASES), solution enhanced dispersion by supercritical fluids (SEDS), and particles from gas-saturated solutions/suspension (PGSS) (Hu et al., 2004; Jung & Perrut, 2001; York, 1999). In the RESS method, the drug of interest is dissolved in supercritical

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fluid. The supercritical solution with dissolved solute is then rapidly expanded through a heated nozzle to ambient condition, leading to extremely rapid nucleation of drug particles. Hence, fine particles with narrow size distribution are obtained. The RESS process could eliminate the drawbacks of the conventional micronization techniques. The high supersaturation ratios (the ratio of the solute mole fraction to equilibrium mole fraction at the given temperature and pressure) and the homogeneous conditions attained due to the rapid expansion of a highly compressible supercritical mixture are distinctive features of the RESS process. The high supersaturation ratios lead to the formation of very fine particles and homogeneous conditions provide narrow particle size distribution. These advantageous features of the RESS method are utilized to produce fine particles of various poorly water soluble drugs; for example, ibuprofen (Kayrak, Akman, & Hortaçsu, 2003), carbamazepine (Gosseelin et al., 2003), aspirin (Huang, Sun, Chiew, & Kawi, 2005), tolbutamide (Shinozaki et al., 2006), and cyclosporin (Young et al., 2000). Furthermore, near-critical fluid micronization has been used for stabilized protein, antibiotics, and anti-virals (Sievers et al., 2007)

Dihydroartemisinin (DHA) is a potential antimalarial drug especially against the tolerant protozoa parasite, *Plasmodium falciparum*, which is the most deadly type of human malaria infection (Titulare, Zuidema, & Lugt, 1991; Wilairatana et al., 1998). DHA is an active metabolite of artemisinin and its derivatives, including arteether, artemether, artelinic acid, and sodium artesunate. It is an endoperoxide containing sesquiterpene lactone structure (Figure 1) which can be synthesized from artemisinin obtained from the Chinese medicinal herb Qinghao (*Artemisia annua* L.) in less step and lower cost than other artemisinin derivatives. Because of high effectiveness in the treatment of malaria caused by multi-drug-resistant strains of *P. falciparum* (Titulare et al., 1991), DHA has received much attention in antimalarial drug development. However, DHA possesses poor water solubility (0.168 mg. mL^{-1} at 30ºC) (Sethabouppha, 1999), leading to a problem in obtaining a good bioavailability dosage form. It was postulated that size reduction of DHA would increase surface area, leading to enhanced dissolution rates. The size reduction was thus a method of interest in this study. Micron to sub-micron particles of DHA may be hardly obtained by mechanical grinding method in which particle size is reduced from large to small. This led to the use of an alternative method, RESS, in which fine drug particles precipitate from highly saturated solution. In this study DHA fine particles were prepared by RESS method using supercritical CO_2 as the solvent. The micronization of DHA by RESS was performed in comparison with mechanical grinding using jet mill and vibration rod mill. Effect of RESS processing conditions—including extraction pressure and temperature, pre-expansion pressure and temperature, nozzle diameter, nozzle temperature, and collecting distance—on the size and morphology of the precipitated DHA particles was investigated. Their physicochemical characteristics also were determined by powder X-ray diffraction, thermogravimetric analysis, and differential scanning calorimetry.

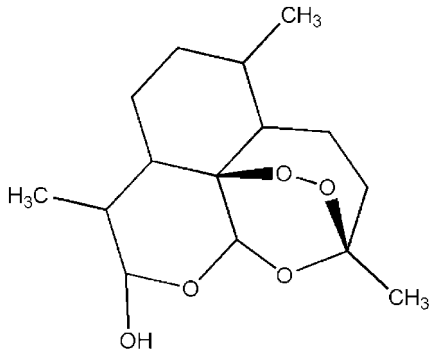

FIGURE 1. Chemical structure of dihydroartemisinin.

MATERIALS AND METHODS

Materials

DHA was purchased from Knoll AG (Liestal, Switzerland). High purity carbon dioxide was used as the solvent. All other reagents were of analytical grade.

Preparation of Ground DHA

DHA was ground by jet pulverizer (Micron-Master® Model 08–506, The Jet Pulverizer Co., Ltd., Palmyra, NJ) and the obtained powder was used as starting material in RESS process. The starting material was further ground by a vibrating rod mill (TI-200, Heiko Seisakusho, Tokyo, Japan) at room temperature for different grinding times up to 120 min. The samples obtained were designated as ground sample. The vibration rod mill operates on the principle of attrition induced by aluminum oxide balls in a mill body. The ground samples were collected, placed in a vial, and kept in desiccator at room temperature prior to characterization.

RESS Apparatus Setup

A schematic diagram of the experimental RESS apparatus (Supercritical sprayer, Nikkiso Co. Ltd, Tokyo, Japan) is shown in Figure 2. The setup mainly consists of a supercritical fluid delivery, an extraction unit, and an expansion unit. The maximum tolerable pressure and temperature of the whole system were 29 MPa and 80°C, respectively. Drug powder was loaded into a temperature-controlled extraction vessel (internal volume, 90 mL). High purity liquid CO_2 was compressed and heated to above the critical pressure and temperature, 7.4 MPa and 31°C, and then introduced to the extraction vessel by a pump (NP-AX-403, Nihon Seimitsu Kagaku Co., Ltd., Japan) at constant flow rate. In each experiment, the amount of CO_2

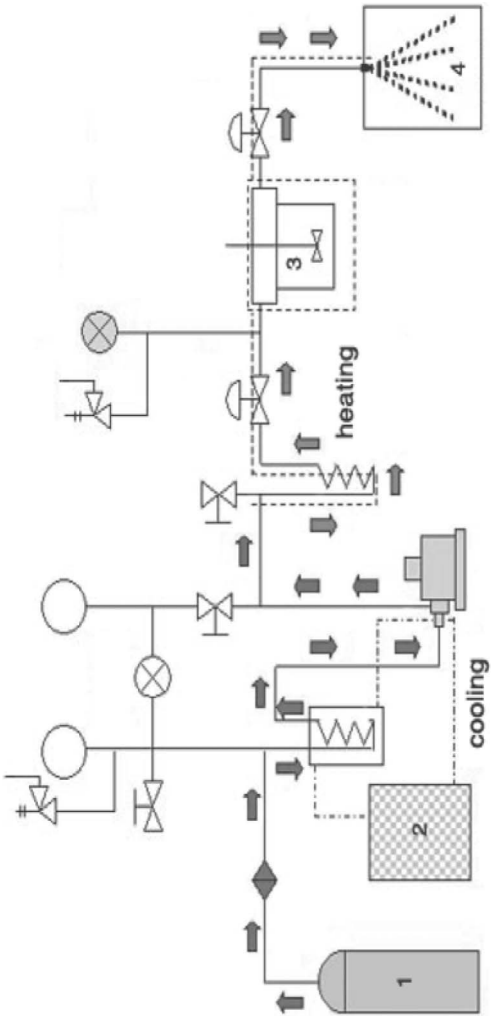

FIGURE 2. Schematic illustration of the rapid expansion of supercritical solutions (RESS) experimental apparatus: (1) CO_2 cylinder, (2) temperature control unit, (3) extraction vessel, (4) expansion chamber.

used was approximately equivalent to 0.2 mole. The drug was kept under supercritical CO_2 at a constant pressure and temperature while magnetic stirring (PC-420, Corning Inc., NY) occurred. After extraction, the drug/supercritical CO_2 mixture was sprayed through a heated nozzle (tungsten carbide spray tip, Unijet®, Spraying Systems Co., Ltd., Japan) into an expansion chamber (inner volume, 12 L) that was held at ambient pressure and temperature. In the expansion chamber, rapid change of the supercritical CO_2 solution into gas phase induced high saturation of the solute and resulted in the precipitation of fine particles. The CO_2 gas was exhausted by using an air compressor (0.75LP-7ST, Hitachi, Japan) and the flow rate was adjusted with ejector cock to collect precipitated particles on a 0.2 μm glass fiber filter (Millipore Corp., MA) placed on the bottom flange of the expansion chamber.

Solubility of DHA in Supercritical CO_2

Two grams of drug in a glass vial was set at the extraction unit. The drug was kept at supercritical CO_2 atmosphere at pressure of 18 and 26 MPa, and temperature of 32 and 60ºC for 3 h. The drug/supercritical CO_2 solution was sprayed into ethanol. Amount of drug was determined spectrophotometrically at 214 nm (UV-160, Shimadzu Corp., Kyoto, Japan). Mole fraction of CO_2 was calculated using a software program based on Lee-Kesler equation. Solubility of drug in supercritical CO_2 was expressed as mole fraction of drug which was calculated from the molar of drug in comparison with molar of drug and CO_2.

Preparation of DHA Particles by RESS

One gram of drug was filled in a glass vial covered with Milliwrap (Millipore, MA, USA) and placed in the extraction vessel. Temperature-controlled CO_2 was delivered into the extraction vessel at constant flow rate (40 mL/min). The drug was kept under supercritical CO_2 at varied pressure and temperature for 3 h. After extraction, the drug/supercritical CO_2 mixture was sprayed through a heated nozzle into an expansion chamber. After repeating the spray at a definite time intervals for 100 times, the fine particles obtained on the filter were collected. The collected particles were kept in desiccator at room temperature prior to characterization. The processing conditions were varied, as shown in Table 1.

Particle Size Analysis

The sample was dispersed in distilled water with Tritron-X as a dispersing agent and sonicated at room temperature. Volumetric particle size distribution of each sample were then determined with laser diffraction on a Microtrac FRA (Nikkiso, Tokyo, Japan; measurement range, 0.1–700 μm) or dynamic light scattering method using a Microtrac UPA® 150

TABLE 1
Solubility of Dihydroartemisinin in Supercritical CO_2

Extraction Pressure (MPa)	Extraction Temperature (°C)	Pre-expansion Pressure (MPa)	CO_2 Amount[a] (mole)	Solubility in Supercritical CO_2 (Mole Fraction, $\times 10^{-4}$)
26	60	19	0.1918	4.42
26	32	14	0.1863	3.44
18	60	15	0.1894	4.65
18	32	11	0.1773	nd[b]

[a]Amount of CO_2 was calculated using software program based on Lee-Kesler equation.

[b]Amount of dissolved DHA was very low and could not be determined.

(Nikkiso, Tokyo, Japan; measurement range, 0.003–6 μm). Mean of three determinations was reported. Particle size distribution was calculated from an equation; (d84%-d16%)/2, where d84% and d16% represent diameters at 84 and 16 cumulative percent frequency undersize, respectively.

Scanning Electron Microscopy (SEM)

The samples were fixed on the stub with conductive double-sided adhesive tape and sputtered with Au-Pd (6:4) by an ion sputter (JEC-550, Jeol, Co., Ltd., Tokyo, Japan) in an argon atmosphere. Particle morphology of DHA was then imaged by using SEM (S-4300, Hitachi Co., Ltd., Ibaraki, Japan).

Powder X-Ray Diffraction (PXRD)

Powder X-ray diffraction patterns were measured by using diffractometer (Miniflex, Rigaku Co., Ltd., Tokyo, Japan). The measurement conditions were as follows: target, CuKα; filter, Ni; voltage, 30 kV; current, 15 mA; scanning speed, 4 º/min.

Differential Scanning Calorimetry (DSC)

A differential scanning calorimeter (DSC6200, Seiko Instruments, Inc., Chiba, Japan) was used. The measurement conditions were as follows: crimped aluminum pan; sample weight, 4 mg; heating rate, 5ºC/min; nitrogen gas flow, 60 mL/min.

Thermogravimetric Analysis (TGA)

A thermogravimetric analyzer (TG/DTA 6200, Seiko Instruments Inc., Chiba, Japan) was used. The measurement conditions were as follows: sample weight, 4 mg; heating rate, 5ºC/min; nitrogen gas flow, 60 mL/min.

RESULTS AND DISCUSSION

Solubility of DHA in Supercritical CO_2

The requirement for micronization by RESS method is that the drug has to be dissolved in supercritical CO_2 prior to expansion of the mixture of drug and supercritical CO_2 (de Castro et al., 1994; Hu et al., 2004; Jung & Perrut, 2001; York, 1999). Thus, the solubility of DHA in supercritical CO_2 could determine the possibility in preparation of drug fine particles by RESS method. The solubilities of DHA in supercritical CO_2 at different extraction and expansion conditions are shown in Table 1. At extraction temperature of 60ºC, the solubilities of DHA in supercritical CO_2 at extraction pressure of 26 and 18 MPa were 4.42×10^{-4} and 4.65×10^{-4} mole fraction of DHA, respectively. The results indicated that DHA had relatively high solubility in supercritical CO_2 when compared with other drugs, for example, tolbutamide (6.89×10^{-5} mole fraction), barbital (3.57×10^{-5} mole fraction), and ibuprofen (11.30×10^{-5} mole fraction), that could be micronized by RESS process (Shinozaki et al., 2006). Thus, application of RESS method in preparation of DHA fine particles seems to be possible as DHA could dissolve in supercritical CO_2.

The results indicated that solubility of DHA in supercritical CO_2 was dependent on extraction pressure and temperature (Table 1). Drug solubility in supercritical fluid is dependent on vapor pressure of drug, drug-CO_2 interaction, and CO_2 density (Baldyga, Henczka, & Shekunov, 2004). Drug vapor pressure is a function of temperature and the density of the supercritical fluid is markedly dependent on the pressure and temperature. Saturated drug concentration in supercritical CO_2 was thus affected by both CO_2 density and drug vapor pressure. The net effect of these two competing factors determines the drug solubility in supercritical CO_2 (Huang, Lu, Kawi, & Chiew, 2004).

At extraction temperature of 32°C, the drug solubility at extraction pressure of 18 MPa was very low, whereas it was obviously increased as extraction pressure was increased from 18 to 26 MPa. However, the effect of extraction pressure on the drug solubility was not clearly observed at the extraction temperature of 60°C and only minimal change in the solubility was observed. At given extraction temperature, an increase in extraction pressure causes an increase in supercritical CO_2 density and dissolving power of CO_2, and subsequently enhances drug solubility in supercritical CO_2. The operation of RESS process at 32°C, which is closed to critical temperature of supercritical CO_2 (31.1ºC), provides the greatest changes in CO_2 density as temperature and/or pressure are slightly changed (de Castro et al., 1994). Thus, the increase in drug solubility with increasing extraction pressure at 32°C was due to a great increase in CO_2 density.

Furthermore, the drug solubility increased with increasing extraction temperature. The effect of extraction temperature on the drug solubility at low extraction pressure (18 MPa) was found to be much greater than that at high extraction pressure (26 MPa). These results indicated that the drug solubility would be changed at a lesser extent when extraction pressure was changed at high extraction temperature or when extraction temperature was changed at high extraction pressure. At given extraction pressure, increasing extraction temperature led to a decrease in supercritical CO_2 density and a concurrent increase in drug vapor pressure (de Castro et al., 1994). The decrease of supercritical CO_2 density caused a decrease of the solvent strength. On the other hand, the concurrent increase in drug vapor pressure is responsible for an increase in drug solubility. It was suggested that the increased drug vapor pressure had dominant effect, thus the increased solubility of DHA with increasing of extraction temperature was obtained at both extraction pressures of 18 and 26 MPa. Furhermore, such change took place at a higher extent at low extraction pressure.

Effect of RESS Processing Conditions on Particle Size and Morphology

The starting material DHA, which was prepared by grinding of the received material with jet mill, had mean particle diameter and size distribution of 7.3 and 3.7 μm, respectively (Table 2).

TABLE 2
Effect of Processing Conditions by Rapid Expansion of Supercritical Solutions (RESS) Method on Particle Size of Dihydroartemisinin (DHA)

Sample	Run No.	Extraction Pressure (MPa)	Pre-expansion Pressure (MPa)	Extraction–Pre-Expansion Temperature (°C)	Nozzle Temperature (°C)	Collecting Distance (cm)	Nozzle Diameter (mm)	Particle Size (μm)	
								Mean	Size Distribution
Starting material[a]		–	–	–	–	–	–	7.3	3.7
Ground[b]		–	–	–	–	–	–	7.2	4.5
	1	18	11	32-32	30	30	0.33	1.2	0.2
	2	18	15	60-60	30	30	0.33	0.9	0.8
	3	26	10	60-60	30	30	0.33	1.1	0.9
	4	26	10	60-60	60	30	0.33	1.1	1.4
	5	26	10	60-60	60	14	0.33	1.0	0.8
RESS	6	26	15	40-40	30	30	0.33	1.0	0.7
	7	26	17	50-50	30	30	0.33	1.6	1.7
	8	26	19	60-60	30	30	0.33	1.7	1.4
	9	26	19	60-60	30	30	0.23	0.8	0.7
	10	26	19	60-60	40	30	0.33	2.4	2.4
	11	26	19	60-60	60	30	0.33	0.9	0.6

[a]DHA from Knoll AG was ground by jet pulverizer prior to use as starting material of the RESS process.
[b]The jet-milled DHA was ground by a vibration rod mill for 15 min.

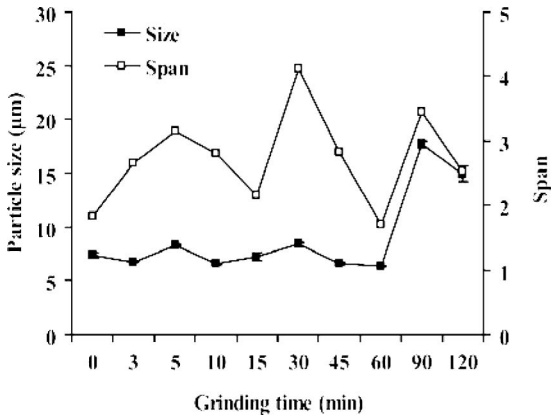

FIGURE 3. Effect of grinding time on mean particle size of dihydroartemisinin.

Grinding of the starting material DHA by vibration rod mill for 60 min did not cause a significant change in particle size reduction (Table 2, Figure 3). Moreover, the drug particles tended to agglomerate and greater particle size was observed as grinding time was further increased to 90–120 min. These results revealed that particle size of the drug could not be reduced by additional mechanical grinding with vibration rod mill.

The RESS-processed DHA had mean particle size of 1–2 μm and narrower size distribution than both jet-milled and ground DHA (Table 2). SEM photomicrographs also showed that the size of RESS-processed DHA particles was smaller than that of both jet milled and ground DHA particles (Figure 4). It was observed that some of the RESS-processed particles were nearly spherical. The difference in particle shape was attributed to the process used. In mechanical grinding method, the large particles are crushed to small particles by forces, resulting in irregular shape, rough surface, and broad size distribution. In RESS process, the drug particles precipitate from supersaturated solution; therefore, under controlled conditions the small and nearly spherical particles with narrow size distribution could be obtained.

The results showed that the RESS processing conditions, extraction conditions, pre-expansion conditions, nozzle diameters, nozzle temperatures, and collecting distances had an effect on mean diameter and particle size distribution of the obtained DHA particles (Table 2). The mean particle size of about 1 μm with narrow size distribution was obtained at extraction pressure of 18 MPa and extraction temperature of 32°C (Table 2; Run no. 1). Other RESS conditions produced the drug particles with mean particle size of 1–2 μm and rather broad size distribution. These results indicated that the operation of RESS process closed to the critical temperature of supercritical fluid could produce small drug particles with narrow size distribution. The effect of each RESS processing parameter on particle size of DHA is discussed as follows.

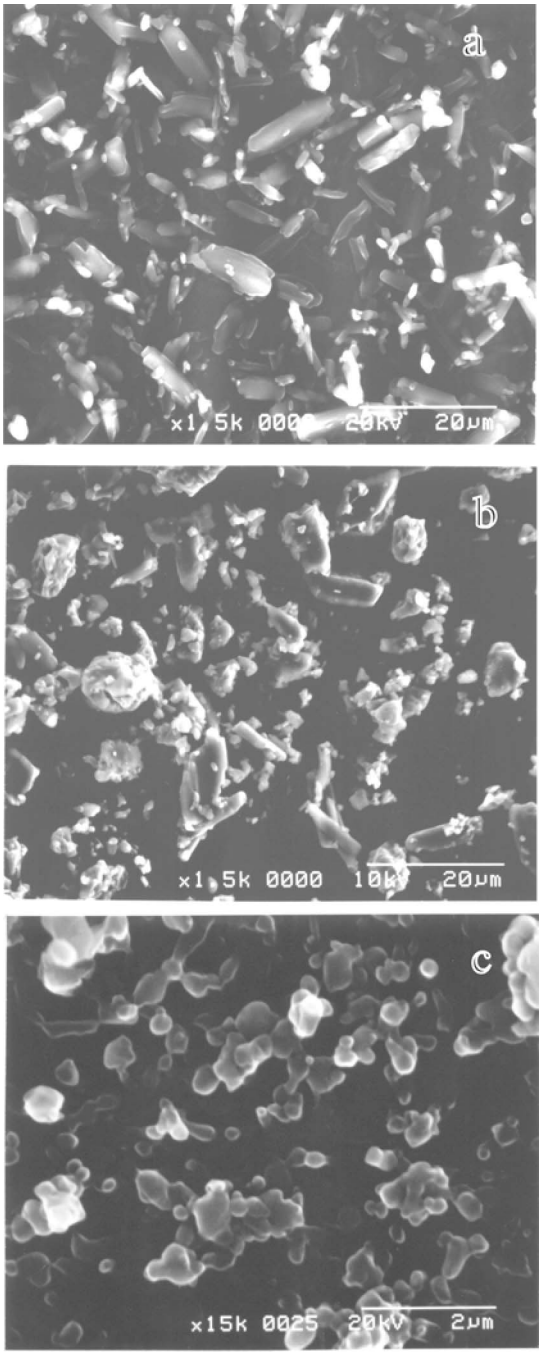

FIGURE 4. Scanning electron photomicrographs of dihydroartemisinin particles. (a) starting material (jet milling); (b) ground for 15 min by a vibration rod mill; (c) RESS-processed at extraction pressure of 26 MPa, pre-expansion pressure of 19 MPa, extraction and pre-expansion temperature of 60°C, nozzle temperature of 30°C, and nozzle diameter of 0.23 mm.

Effect of Extraction Condition

In this study at extraction temperature of 60°C, the size of DHA particles appeared to increase as extraction pressure increased from 18 to 26 MPa (Table 2; Runs no. 2 & 8). According to the classical theory of nucleation, higher supersaturation causes higher nucleation rate and the particle volume is inversely proportional to the nucleation rate. Thus, smaller particles are obtained at 18 MPa as a result of higher drug solubility in supercritical CO_2 (Table 1). Moreover, particle formation in expansion unit might be resulted from a decoupling of two processes of nucleation and particle growth. Therefore, at 26 MPa the particle growth may be dominant or particle may consist of several nuclei during the growth process (Huang et al., 2005). Therefore, larger particles seemed to be readily produced and a broad particle size distribution was obtained.

At extraction pressure of 18 MPa, the particle size of DHA decreased as extraction temperature increased (Table 2; Runs no. 1 & 2). The increase of extraction temperature induced the increase of solubility of DHA in supercritical CO_2 (Table 1). This causes an increase in supersaturation of drug in supercritical CO_2 at higher extraction temperature. The higher supersaturation ratio led to the higher nucleation rate, thus a smaller size of drug particles was obtained.

Effect of Pre-Expansion Condition

The RESS processes with different pre-expansion pressures ranging from 15 to 19 MPa and pre-expansion temperatures ranging from 40 to 60°C were performed at the related extraction temperature to control amount of supercritical CO_2 as 0.19 mole. Mean particle size of the precipitated particles obtained from different pre-expansion conditions was in the range of 0.7–1.7 µm (Table 2; Runs no. 6, 7, & 8). Furthermore, mean particle size of the precipitated particles tended to increase with increasing pre-expansion pressure and temperature (Table 2; Runs no. 6, 7, & 8). It was explained that an increase of pre-expansion temperature led to an unsaturated solution and resulted in a decrease of the supersaturation ratio, thus inducing the formation of larger particle size. Similar results were found in case of naphthalene (Liu & Nagahama, 1996), salicylic acid (Reverchon, Donsi, & Gorgoglione, 1993; Yildiz, Tuna, Döker, & Çalimli, 2007), benzoic acid (Helfgen, Türk, & Schaber, 2000), and titanocene dichloride (Wang, Chen, & Yang, 2005). However, the contrary results were reported in case of ibuprofen (Kayrak et al., 2003). Huang and coworkers (Huang et al., 2005) have reported that pre-expansion temperature has little effect on particle size of aspirin. Therefore, the effect of pre-expansion temperature on particle size might be dependent on the nature of solutes and the interaction between solute and solvent at process conditions (Yildiz et al., 2007).

Effect of Nozzle Diameter

The results showed that nozzle diameters (0.23 and 0.33 mm) had an effect on the particle size of the drug particles (Table 2; Runs no. 8 & 9). Smaller particle size was obtained when smaller nozzle diameter was used. The decrease in particle size with decreasing of nozzle diameters might be attributable to smaller droplet size formed by using smaller

nozzle diameter. However, there were reports that nozzle diameter did not affect the size and morphology of precipitated particles in case of aspirin (Huang et al., 2005) and napthalene (Liu & Nagahama, 1996; Mohamed, Debenedetti, & Prud'homme, 1989; Wang et al., 2005).

Effect of Nozzle Temperature

The effect of nozzle temperature ranging from 30 to 60°C on the size of drug particles was investigated at fixed extraction pressure and extraction temperature of 26 MPa and 60°C, respectively. At pre-expansion pressure of 10 MPa, nozzle temperature did not significantly affect size of the precipitated particles (Table 2; Runs no. 3 & 4). However, at pre-expansion temperature of 19 MPa, increase of nozzle temperature from 30 to 40°C caused a slight increase in particle size and a further increase of nozzle temperature from 40 to 60°C led to a decrease in particle size (Table 2; Runs no. 8, 10, & 11). These results suggested that particle size of DHA particles tended to decrease with increasing nozzle temperature. This was affected by the difference between pre-expansion temperature and nozzle temperature. The lower nozzle temperature induced a greater difference in temperature between pre-expansion and nozzle, resulting in early nucleation of drug prior to expansion. The early nucleation led to the formation of larger drug particles. It was suggested that the effect of nozzle temperature on particle size was dependent on extraction and pre-expansion conditions. In this study, the effect could be observed at extraction pressure of 26 MPa, extraction temperature of 60°C, and pre-expansion pressure of 19 MPa.

Effect of Collecting Distance

The collecting distance between spray nozzle and collecting frit was varied from 14 to 30 mm. The results indicated that longer collecting distance slightly increased the size of the precipitated DHA particles (Table 2; Runs no. 4 & 5). Similar results were found in RESS processing of caffeine (Ksibi, Subra, & Garrabos, 1995) and ibuprofen (Kayrak et al., 2003). In contrast to this study, increase of spray distance reduced the particle size of salicylic (Reverchon et al., 1993) and ibuprofen (Charoenchaitrakool, Dehghani, Foster, & Chan, 2000).

Particles are generated by nucleation process due to an increase of the supersaturation ratio during expansion. Particle growth by coagulation must be allocated to the flow field outside the nozzle and supposedly in the zone closed to the nozzle exit. The time for particle growth is decreased when the residence time of all particles is minimized inside of the expansion chamber. It was suggested that collecting distance affected the nucleation of particles during expansion process. It could be explained that when the spraying distance is short, the time that the particles stay in the growth region also is short. As shorter times were given to the particles to spend in the expansion region before reaching the collection surface, particle growth is avoided (Kayrak et al., 2003; Ksibi et al., 1995).

Physicochemical Properties of RESS-Processed DHA Particles

Crystalline Characteristics

Powder X-ray diffraction pattern of jet milled DHA showed the three major diffraction peaks at angles (2 θ) of 7.7, 9.3, and 11.1, respectively (Figure 5). Grinding by vibration rod mill for 15 min did not cause a significant change in powder X-ray diffraction pattern. The RESS-processed particles also showed characteristic diffraction pattern but the diffraction peak intensity was slightly lower when compared to the jet milled and ground DHA. The results revealed that the crystalline DHA particles were obtained from RESS processing. It was suggested that the slight decrease in diffraction peak intensity was probably due to a reduction in particle size or a transformation of small fraction of DHA from crystalline to amorphous phases.

Thermal Behavior

Change in thermal behavior of DHA particles is presented in Figure 6. For jet-milled DHA, DSC thermogram shows the exothermic peak with the onset temperature of 131°C. Weight loss in TGA thermogram of jet milled DHA was observed in the same temperature range of the exothermic peak presented in DSC thermogram (data not shown). This exothermic peak was attributed to melting followed by thermal decomposition. DHA undergoes thermolysis and gives decomposition products including desoxyartemisinin and a preponderant decomposition

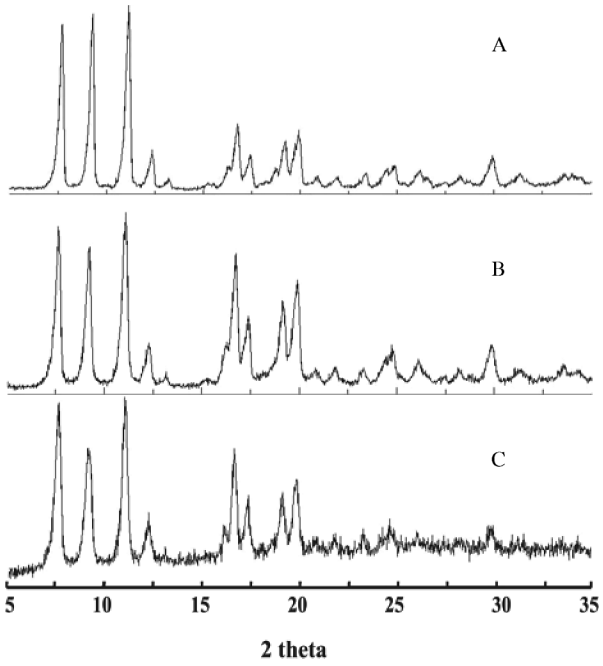

FIGURE 5. PXRD patterns of dihydroartemisinin particles. (A) starting material (jet milling); (B) ground for 15 min by vibration rod mill; (C): RESS-processed at extraction pressure of 26 MPa, pre-expansion pressure of 19 MPa, extraction and pre-expansion temperature of 60°C, nozzle temperature of 30°C, and nozzle diameter of 0.23 mm.

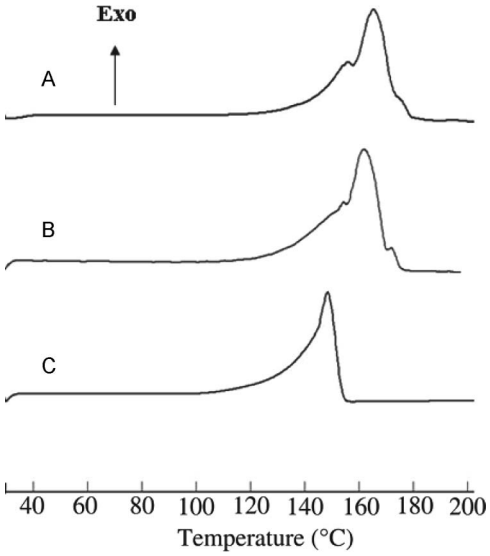

FIGURE 6. DSC thermograms of dihydroartemisinin particles. (A) starting material (jet milling); (B) ground for 15 min by vibration rod mill; (C) RESS-processed at extraction pressure of 26 MPa, pre-expansion pressure of 19 MPa, extraction and pre-expansion temperature of 60°C, nozzle temperature of 30°C, and nozzle diameter of 0.23 mm.

product consisting of 2 epimers; 4a, (2S, 3R, 6S)-2-(3-oxobutyl)-3-methyl-6-[(R)2-propanal]-cyclohexanone; and 4b, (2S, 3R, 6R)-2-(3-oxobutyl)-3-methyl-6-[(R)2-propanal]-cyclohexanone (Lin, Theoharides, & Klayman, 1986). The onset temperature of exothermic peak of the RESS-processed DHA was slightly lower than that of the unprocessed DHA. It seemed that this change in thermal behavior was probably a result of particle size reduction or a decrease in crystallinity.

CONCLUSION

The crystalline DHA fine particles were successfully produced by RESS method. The size of RESS-processed particles was dependent on processing conditions including extraction condition, pre-expansion condition, nozzle diameter, nozzle temperature, and collecting distance. The particle size of drug was related to the solubility of drug in supercritical CO_2 at each processing condition. The fine particles of DHA (about 1 μm) with narrow size distribution could be obtained at extraction pressure of 18 MPa and extraction temperature of 32°C, which is closed to the critical temperature of supercritical CO_2. The RESS method produced smaller drug particles when compared with mechanical grinding method. The smaller particles of RESS-processed DHA would have higher surface area, resulting in higher drug dissolution and bioavailability.

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